Newsletter for October 2024

Integument review progress on feathers

By Lathreas, Tiltwolf, and Zennith
October 30, 2024

In the Integument Review Project, we are doing deep-dive literature research to get ourselves ready for determining what it would take to grow fur, scales, or feathers on a person's skin. We have already covered the basics of fur and scales in previous updates, and now we are focused on feathers to unravel their mysteries.

Feathered skin is the most complex type of integument that we are reviewing. Feather structures are finely detailed, interlocking with each other in various ways, and serve an incredible diversity of physiological functions across species. The literature is somewhat laborious to navigate and frequently incomplete: many of the more mechanistic papers are more interested in contour or flight feathers than downy feathers, but we need to understand both (and all the other kinds of feathers).

Nevertheless, we're getting close to getting our feather chapter out! This publishing pattern is similar to what we're doing for the other integument type. Each integument type is getting a basic characterization/descriptive chapter (what the integument *is composed of* on an animal), then a chapter of how it develops in embryogenesis and molecular signaling details, then one or two chapters for our ideas for engineering the structures, as well as how we can fill in any scientific gaps that need resolved.

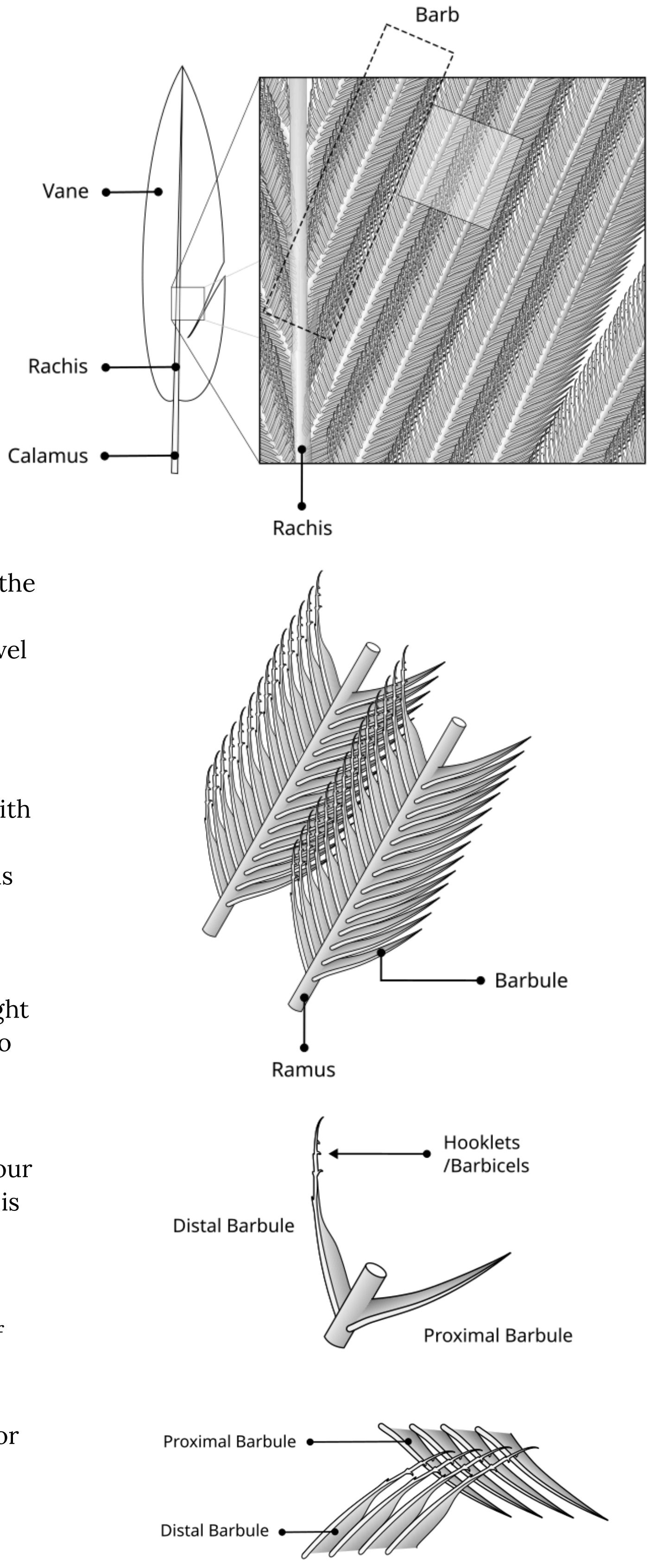


Figure 1: *Key structures on a feather*

Microstructure of a contour feather

The most recognizable “feather” of all is probably the *contour feather*, which is likely the type of feather you would pick up when you see it on the ground. This type of feather covers the entire body of a bird, and is used in both flight and insulation. Structurally, it is stiff, strong, and highly sophisticated, making it one of the most challenging types of integumentary structures to engineer. This also makes it one of the most exciting ones to put under a magnifying glass. And with the knowledge that most other feather types are going to be less structurally intricate, it forms an excellent example to understand better what a feather really is, and what it would take to engineer them.

Before we dive into the nitty gritty of how feathers really work, it is important to get the terminology out of the way. You will recognize a feather’s *stem* and *vane*: the stem is the central shaft that holds a feather up, and the vane is the smooth foil created by tiny fibers that sit on either side of the stem. How the vane holds together and can be pulled apart and back together is downright fascinating, and requires a very intricate hierarchically branched microstructure to properly work.

In the most simplified form, contour feathers consist of a single stem, a first layer of branches called *barbs*, a second layer of branches called *barbules*, and, in some barbules, a third layer of branches called *barbicels*, which is the final layer of branching. Each of these branches are very evenly spaced out, to form the smooth vane of a feather. When barbicels are absent, the feather no longer is able to interlock adjacent barbs, resulting in fluffier, less rigid feathers, such as downy feathers.

The stem of a feather is called the *calamus* at the base, and *rachis* at the point where the vane starts. This stem appears to be about ten cells thick, from what we could see on light microscope images. The stem of a barb is called the *ramus*, and is give or take three cells thick in at least one of the samples we checked. Barbules consist of carefully stacked cells, and come in two types, displayed in Figure 1: distal barbules, which are those that point away from the skin of the bird, and proximal barbules, which are those that point back towards the skin of the bird. The distal barbules contain barbicels, shaped like hooklets, which grab onto the proximal barbules like velcro. The proximal barbules, in turn, do not have any hooklets, but are shaped like a ridge for the distal barbules to grab onto.

Various modifications to the above structures - the rachis, the barbs, barbules, and the barbicels (if present) - are ultimately responsible for all the various shapes that feathers can take on. So, in order to engineer feathers, it will be very important to understand how the properties of those structures are determined.

The biological processes that produce the complex shapes of feathers are, as you might imagine, complex. We’ve mentioned some of this in our April 2024 newsletter (look under the “Feather growth” heading). Our summary of feather growth is currently in the feather embryogenesis/molecular signaling chapter, since it’s intensely mechanistic.

Structural molecules

Let’s zoom in a little bit. We have also been looking at the proteins that physically make up feathers, and how they contribute to their structure.

Feathers, like hair, are composed primarily of structural proteins that contribute to their durability. However, in addition to the alpha keratins that they share with hair, they possess another kind of protein with even higher durability.

These have historically been called “beta keratins”, but more recent literature has switched to calling them keratin-associated beta proteins (KABPs), corneous beta proteins (CBPs), or beta proteins, since they don’t seem to share any actual homology with alpha keratins. Interestingly, KABPs are even more abundant in mature feathers than the classical alpha keratins, and it is believed that the alpha keratins may actually only be there to help guide KABP assembly during development. It's therefore very important for us to consider the structural and functional properties that KABPs impart into the feather in order to faithfully reproduce it.

Alpha keratins and beta proteins both function by heavily crosslinking to each other, and they do this via disulfide bridges, forming a dense and rigid protein matrix inside the cells. The degree of crosslinking controls the hardness of the material, and it is, in turn, controlled by the amount of cysteine residues in a particular keratin molecule. There are dozens of these genes with varying amounts of cysteine, and indeed, in humans, we see a strong correlation between cysteine content and material hardness. For example, in humans, epithelial keratins expressed in skin have a comparatively low number of cysteines (0-14 per molecule; 0-3 per 100 residues), whereas hair keratins have far more (20-34 per molecule; 5-8 per 100 residues). It is thus common to refer to alpha keratins as being either "hard" or "soft" based on cysteine abundance.

KABPs – beta proteins – are even more heavily crosslinked. While the actual number of KABPs is lower, the relative number when adjusting for protein length is much higher due to their smaller size. In chickens, feather KABPs range from 6-8 cysteines per 100 residues, and the same number per molecule, as they are also about 100 residues long. Furthermore, their cysteines are concentrated in "hot spots" near each end of the KABP molecule, with most being in the N-terminal domain and 1-2 being near the C-terminus to allow end-to-end linkage. The middle region, which is not crosslinked, may function to provide some elasticity, increasing the amount of stress that a feather can withstand before breaking.

The exact structure of the crosslinked KABP matrix in feather cells is not known, primarily because KABPs are notoriously difficult to solubilize, making it difficult to do classical protein structure experiments like X-ray crystallography or NMR. We can glean some information from intact feather structure experiments, such as IR spectroscopy, which tells us that there must be antiparallel beta sheets within the structure. Based on simulations with CHARMM, a structure prediction program developed before the advent of AlphaFold, this has been previously interpreted to mean that KABPs adopt a mostly globular (spherical) structure (Calvaresi et al., 2016). However, the data is also consistent with parallel sheets of elongated KABPs that are then stacked in an antiparallel fashion. This brings us to some structural predictions we have found when running chicken feather keratin 1 through the AlphaFold3 model.

The AlphaFold prediction suggests that KABPs may be organized as parallel elongated strands such that the beta sheets match up with each other. The model tries to regress the structure into a roughly globular shape by using the flexible regions between the beta strands to create bends in the sheets, but in our opinion, this is mostly due to the AlphaFold model inherently disliking elongated structures. We have also observed the model trying to bend alpha keratins in half to reduce the total length, and the structure is almost exactly correct in comparison with experiments, except for that bend.

We have also tried versions where we force the model to work with end-to-end joined dimers of feather keratin 1, and this does help to increase total length, although the model still tries to regress it to fit into a sphere.

To date, there is no solved structure from experiment, so either of these predictions could be correct - globular antiparallel structures predicted by Calvaresi et al. using CHARMM, or parallel sheets stacked in an antiparallel way based on our AlphaFold3 predictions. To ensure good structural integrity of feathers, it may be necessary to ensure that when we express feather keratins artificially in human cells, that they fold and assemble correctly, and it thus becomes important to know which of these is the right answer.

But the good news is, we can find out! In future experiments, when this becomes necessary to know, we propose a set of crosslinking experiments coupled with mass spectrometry to help map the contacts between KABP molecules in feathers. It has already been shown that feather rachis can be solubilized by digestion with the trypsin enzyme under reducing conditions, which breaks the disulfide bonds in addition to cutting up the proteins into smaller pieces. It stands to reason that it may also be possible to do so with trypsin under oxidizing conditions, retaining the disulfide bridges while still cutting the proteins into small enough pieces to work with. Such fragments can then be mapped using LC-MS/MS (liquid chromatography tandem mass spectrometry) to tell us where the crosslinks are and help us determine what the correct pattern of folding is. We can then use the same procedure with human cells expressing recombinant KABPs to determine if they assemble correctly into a similarly durable protein matrix.

Ultimately, answering questions like this will help us engineer the structural proteins necessary to build feathers, and ensure they self-assemble in the correct way during feather growth.

Coloration mechanisms

Birds can be pretty colorful. How does that happen? It's not like they apply make-up or anything crazy like that…

gets handed a card from off-screen

…Correction: Okay so birds can apply make-up too.

The mechanisms that affect the appearance of the integument in birds are incredibly diverse, and without question the most complex and varied ways of accomplishing coloration that we're covering in the Integument Review Project.

Let's talk through a few cases of how coloration is accomplished.

Parakeets

Budgerigars (parakeets) are quite colorful birds. They have feathers that are green, blue, yellow, white, and various tones of grays. The colors are accomplished by a blend of 3 mechanisms: yellow pigment, melanin, and structural coloration (D'Alba et al, 2012).

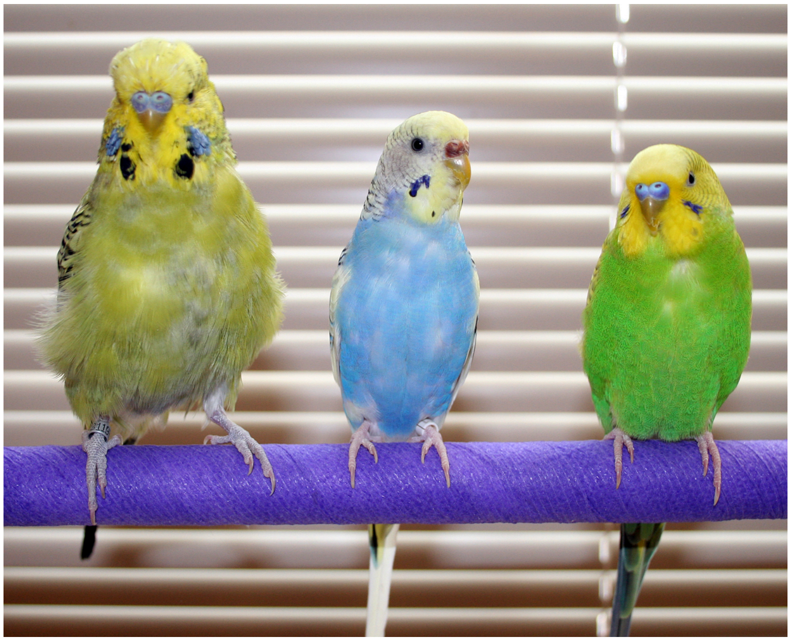


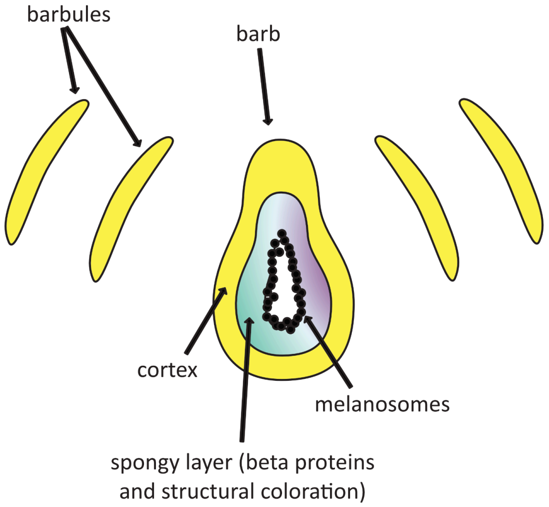
***Figure 2**: Photo of several parakeets showing a variety of colors. Note the middle parakeet has a prominent blue coloration, which is only possible due to structural coloration. Photo is copied from here with gratitude under a CC BY-SA 2.0 license.*

The yellow pigment in some parakeet feathers (psittacofulvin) is naturally synthesized by the birds; it's not a chemical obtained from the diet. More specifically, the pigment is produced in growing feathers (while the feather tissue is still alive), in temporary populations of cells. Those temporary cells secrete pigment that is taken up by nearby cells that become permanent parts of a feather's structure (the barbules as well as the surface of barbs) (D'Alba et al, 2012; Cooke et al, 2018). Once the feather mature and the cells die, no more pigment is produced. The yellow pigment, if present, is in the outermost layer of barbs (the cortex), as well as barbules.

Under the cortex, there is structural coloration. Parakeet feathers, structurally speaking, are largely made of keratin-associated beta proteins (KABPs, and sometimes we also call them "beta proteins"), which form dense, hard polymers. KABPs are especially dense on the outer shell of feather structures (like the cortex of barbs), while the interior is less dense, with many air pockets, saving weight. Structural coloration in these feathers is accomplished by tuning the size of the air pockets: the smallest air pockets (e.g. 87 nanometers) result in purple feathers, whereas larger air pockets (247 nanometers) are used in gray feathers (D'Alba et al, 2012).

Finally, there may be a layer of darkly colored melanosomes, containing melanin. Melanin can be used even in light-colored feathers, and affects the perceived color in non-obvious ways. Melanin is produced by melanocytes in these feathers, and production also ceases upon feather maturity.

Figure 3: *Layering of color-affecting mechanisms in parakeets*



The skin of blue-footed boobies and chickens

Why do blue-footed boobies have such vibrantly colored, blue feet? And why do chickens have yellow feet?

The answer to both questions is that carotenoids are incorporated into their skin. Carotenoids are a class of small, organic molecules that are obtained from the diet. Carotenoids range in appearance from yellow to red. While animals can't synthesize carotenoids directly, animals do modify carotenoid color by some chemical modifications, shifting them from yellow to red or red to yellow.

Chicken feet are yellow, as it turns out, because carotenoids are incorporated into them (Eriksson et al, 2008). As well, the foot color of blue-footed boobies gets less vibrant if their diet is low in carotenoids (Shawkey and D'Alba, 2017; Velando et al, 2006).

But carotenoids aren't blue, right? Right. Enter: our old friend, structural coloration. Here, the structural coloration is happening in the dermis of blue-footed boobies, and is implemented by co-aligned polymers of collagen. Structural coloration gives the birds their famous blue feet, and carotenoids make the blue brighter (Velando et al, 2006).

Carotenoids in and on feathers

Carotenoids are used frequently by birds for feather coloration, as well. In a lot of cases, carotenoids are incorporated directly into feathers during feather growth (Stradi et al, Comp Biochem Physiol, 1995). The mixture of carotenoids can vary from one site on the body to the next, resulting in more yellow or more red pigmentation (or other, different colors in less obvious ways due to interference).

Greater flamingos, however, didn't want to wait on evolution to make their feathers more red with carotenoid incorporation. So, they took matters into their own wings, got some red pigment, and are now, consequently, fabulous (Chiale MC et al, 2021). More seriously, this may help protect the feathers from damage from intense sunlight, and is also used as a sign of fitness in sexual selection.

Overview of coloration

In terms of takeaway messages, these are the most interesting phenomena to keep in mind::

- Pigments (psittacofulvin and melanin) can be synthesized by an animal, or obtained from diet (carotenoids)
- Pigments can be permanently incorporated into the feather's material, or applied after growth
- Structural coloration can be implemented with KABPs and core structural features of the feather, or via collagen in the dermis
- Interaction between pigments and structural coloration affects perceived color of feathers and skin

This has certainly been interesting to learn about! We now have the main concepts of bird coloration figured out, despite how many possible mechanisms there are. We just want to read a few more research articles before being satisfied. We have some questions that include understanding how carotenoids and melanin can be non-evenly distributed within a feather, and we'd also like to look more into how some birds (like hooded vultures) have reddish skin, which is due to blood circulation near the surface.

At the same time, it's worth remembering that the overall intent, here, is to understand what nature does: what biological solutions for coloration in feathers and bird skin already exist. After we've done that homework, we will be able to consider which coloration strategies are most worth pursuing, with a more engineering-focused mindset.

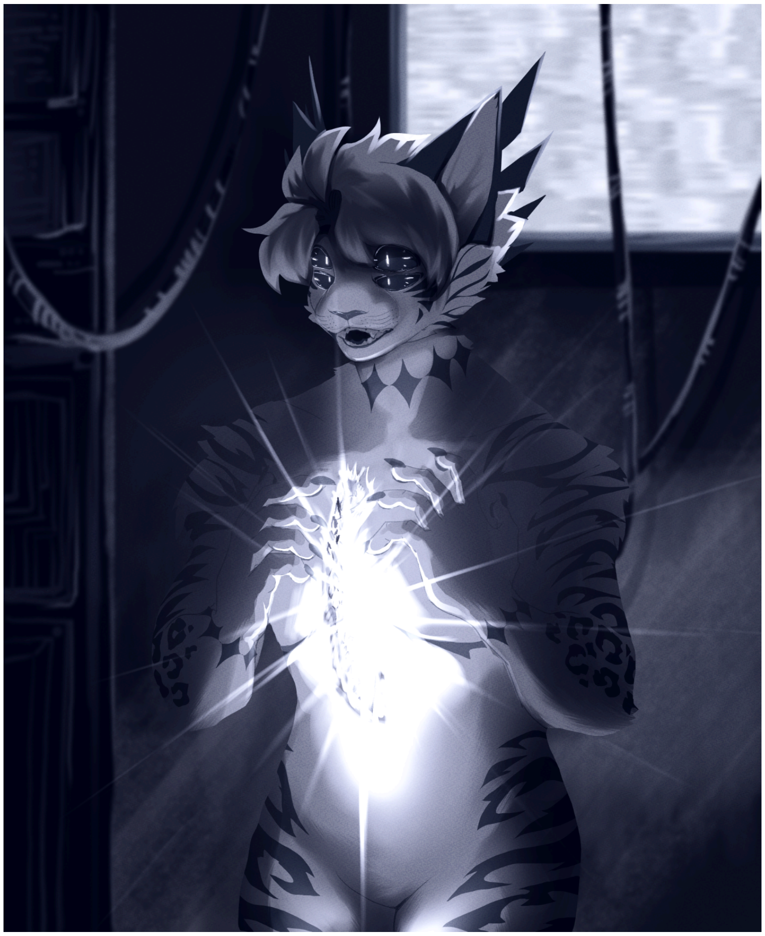
We've got a few more things to do before we post this chapter on feathers to the website - getting close! When we do, it will be posted to https://freedomofform.org/research/project-integument-review/ under "Released chapters".

Thank you as always for reading, and to our supporters on Patreon for helping make all of our work possible!

Volunteer Spotlight: Chronolatry/Lev

By SvarOS | October 27, 2024

Chronolatry, or Lev for short, also known as Kotya or Cold, is an artist and aspiring webcomic creator. They're part of the FFF's social media team and help manage the community as a volunteer moderator.



SvarOS: What's a moderator for the Foundation do?

Lev: The moderation team, including myself, ensures that members of our community are safe and respectful. We work together to stabilize the server environment and ensure it remains a respectable place for discussion.

SvarOS: What do you most enjoy about the foundation's community?

Lev: I enjoy the simultaneously similar and diverse experiences of others' identity and self-expression. There are so many different forms, each unique in its way. For me, as someone who prefers a non-human identity, we share the struggle of needing to change our bodies to reflect ourselves. We all understand and view each other as we intend to be seen.

SvarOS: Before we move on. How would you explain this concept of a non-human identity to someone on the outside looking in?

Lev: I believe it's similar to being transgender and seeking gender-affirming care. Many nonhuman-identifying individuals understand their species identity as a core part of themselves. Much like those who are transgender, they know that their gender identity is a core part of themselves. Both often experience dysphoria due to their body not aligning with their identity (and these two groups overlap at times as well). Morphological freedom encapsulates gender identity and total bodily affirmation, amongst other things. Everyone deserves to have the body they feel most comfortable in.

SvarOS: You also manage some of our social media. What platforms do you manage?

Lev: I only manage one platform for the Foundation, our BlueSky. I stepped up to manage the account because that platform was the easiest for me.

SvarOS: What is involved in managing social media for our organization?

Lev: It involves managing our presence on a particular platform and coordinating with our team to distribute information to the public, such as monthly newsletters. Communication with other team members is essential to delivering refined messages to the world.

SvarOS: What is the most rewarding aspect of running social media for the Foundation?

Lev: I enjoy contributing to the foundation at such a critical time. As someone who firmly believes in morphological freedom, I think the Freedom of Form Foundation will help those like me who struggle with species dysphoria. Helping those folks in mind is fulfilling to me. Knowing I helped even just a little for people like me is fulfilling.

SvarOS: What do you find the most challenging aspect?

Lev: My only difficulty is keeping up with what I need to post. It can get interesting at times.

SvarOS: Circling back to social media. What changes have you seen in the social media landscape? How is it now compared to, say, a few years ago?

Lev: I remember when Facebook, Tumblr, and Twitter were big. Everyone had a page or group on there, regardless of the topic. The same thing with Twitter. They have done their job, but there are new spaces for people to connect. Often, these new environments foster new users and interests, both old and new.

SvarOS: What has been the most exciting development you've seen from the foundation? Any scientific achievements or something similar?

Lev: Oh, I must say I'm torn! The head anatomy video was wonderfully intriguing, and the integument [skin] project developments were fascinating. The video was very in-depth yet friendly to those unfamiliar with anatomical terminology, like me. The integument project's progress excites me as I love having my fur one day with coloration and markings!

SvarOS: What about you? What are you studying or learning?

Lev: I'm currently in community college, studying Japanese foreign language. I originally went for art, but I felt that studying it only hindered my passion for the craft, so I'm now indulging in my interest in the Japanese language.

SvarOS: So, here's something fun as a final note. After all is done and we accomplish our goals, what can you tell me about your ideal form?

Lev: I'm always partial to bits and pieces of other forms, so I suppose I'd be a shapeshifter at heart, which, outside of mind uploading and a small gaggle of robotic bodies, likely isn't feasible. However, the form I'm most partial to, at least in terms of aesthetics, is that of a feline hybrid: a mix of domestic cat, snow leopard, and white tiger. I've always wanted white fur with black markings. I want a few things as an exotic twist, though, with four eyes and a pigmented sclera. May not be feasible but, at this point, I'll take what I can get!